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ISSUES IN MONITORING POPULATION EXPOSURES

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16. ABSTRACT

This manuscript explores the risks associated with exposure to carcinogenic chemicals that have become a growing source of public concern. Steadily increasing numbers of agents are being identified as mutagens and carcinogens. Furthermore, we are increasingly aware of sources of exposure to such agents both in the occupational setting and general environment. There is a growing need for reliable and sensitive methods for detecting human exposure to carcinogenic hazards at the time of their occurrence. By detecting hazards early, significant reduction in the risk of adverse health outcome can be achieved by reduction or termination of exposure.

The monitoring of human populations for genetic damage has the potential to improve our ability to detect health hazards and prevent or reduce the risk of workplace-exposure-related cancer. In the long run this can reduce costs related to illness. It is possible that the costs of exposure control can also be reduced by better establishing the sources of significant exposure and by understanding the human response to exposure. However, its greatest promise is in the protection of human health through the early detection and prevention of disease processes resulting from mutagenic exposure.

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I. Introduction

The risks associated with exposure to carcinogenic chemicals have become a growing source of public concern. Steadily increasing numbers of agents are being identified as mutagens and carcinogens. Furthermore, we are increasingly aware of sources of exposure to such agents both in the occupational setting and general environment. There is a growing need for reliable and sensitive methods for detecting human exposure to carcinogenic hazards at the time of their occurrence. By detecting hazards early, significant reduction in the risk of adverse health outcome can be achieved by reduction or termination of exposure.

The identification of carcinogenic agents and assessment of risk related to exposure to them is based on the use of two methodologies: 1) *in vitro* and animal studies in the laboratory and 2) epidemiological studies in human populations. The detection of human exposures to biologically significant levels of carcinogenic agents is a complex matter which neither of these approaches really addresses.

Although laboratory studies can identify hazards, quantitate dose response effects, and explore mechanisms of action, extrapolation of results to human risk is often questioned. In addition to uncertainties about the similarities of human and animal response, the complexities of human exposure and variability of the human genetic background cannot be duplicated in the laboratory.

Epidemiological studies do reflect human experience but, for use in measuring carcinogenic risk, studies require large populations with well documented histories of exposure and health outcome which are rarely available. Even in those circumstances where good studies can be performed they only identify the hazard after the health of the studied population has been adversely affected. The opportunity for prevention has been missed. Populations experiencing current exposures may face conditions very different from those of earlier studied populations in terms of level of exposure and concomitant exposures so that risk assessment again requires extrapolation.

Both laboratory and epidemiological studies are vital to the detection of carcinogenic chemicals and the evaluation of their risk to man. However, neither approach can identify human exposures to carcinogens as they occur. Reliance on measurement of environmental levels of carcinogens is not a satisfactory means to detect exposure for several reasons. A complete ascertainment of all the hazardous, or potentially hazardous, chemicals in an environment is usually not available. Even if the levels of agents of concern are known, workers may move about the environment in a complex manner which is difficult to document. Integrating the total exposure then becomes difficult. Finally additional exposures due to diet, medications, lifestyle factors or home environment may interact with occupational exposures modifying responses to agents in ways not predicted by animal tests.

For these reasons methodologies for detecting exposures to genetically hazardous chemicals should include tests which measure

biological responses to exposure. In this way all factors contributing to exposure can be integrated. This paper will discuss the use of biological monitoring tests for the detection of human population exposures to genetically toxic agents (including carcinogens), the rationale on which such tests are based and the characteristics of some of those tests. Issues in the design and interpretation of studies using batteries of tests will be considered and, as an example, a recently conducted study of autopsy service workers exposed to formaldehyde will be described.

II. Conceptual Basis for Genetic Monitoring

Exposure to genetically toxic agents initiates a process which is diagrammed in figure 1. Cells receiving a significant dose of the agent will experience damage to DNA. This damage will be resolved in one of three ways. The damage may be lethal resulting in cell death. DNA repair processes may restore the damaged DNA to its original structure resulting in no ultimate alteration in the cell. Alternatively in the course of repair or replication a permanent change in DNA base sequence, or mutation, may occur. The mutagenic event is thought to play a significant role in the process leading ultimately to neoplasia.[1] Recent molecular studies of oncogene sequences have implicated both base substitution mutation, and gene rearrangement in the activation of oncogenes.[2]

Neoplasia appears after a prolonged latency period as an adverse outcome to which the original mutation contributed. In general, this

sequential process is understood to occur in man and animals in fundamentally the same manner. Neoplasia is a relatively rare event. In addition, the long latency of human cancer makes the association of cancers with specific environmental exposures difficult to document. However, various manifestations of the initial genetic damage are observed in a large percentage of treated animals. For example, a chemical may produce tumors in a small percentage of treated animals after a lifetime of exposure, however, the same agent used in acute studies may produce chromosome damage in a high percentage of bone marrow cells immediately following exposure.

The rationale behind the use of genetic damage assays as indicators of exposure is that the events observed are high frequency initial events in a process which ultimately produces neoplastic changes in a small subset of the affected cells. The observation of initial genetic damage in carcinogen treated animals is the rationale for the use of these tests. In addition, accumulating experience indicates that genetic damage is also been observed in human populations exposed to known carcinogens.[3] The types of events associated with carcinogen exposure include chromosome damage (structural aberrations and sister chromatid exchange), DNA or protein adduct formation, somatic mutations in peripheral lymphocytes and elevated frequencies of morphologically abnormal sperm or sperm bearing two Y fluorescent bodies. In addition the presence of mutagens in body fluids, particularly urine, has been associated with several chemical exposures.

These events are simply and rapidly detected in cells and body fluids which can be obtained with a minimum of risk or even inconvenience to the subject. In addition these tests can be performed under properly controlled conditions, with ranges of interindividual and temporal variation consistant with many commonly performed clinical laboratory procedures.[4]

III. Monitoring tests in use

A number of tests have been developed for use in genetic monitoring. Some have been used rather extensively while others are relatively new and not extensively characterized. Different tests can detect events in somatic or germinal cells. Also, events occurring at the molecular and chromosomal levels can be detected. Some of the events are persistant allowing cumulative effects over a period of time to be evaluated. However, other events are transitory so that events associated with specific time periods can be studied. Table 1 classifies several of the tests now in use or in development with respect to these characteristics. The following sections briefly describe the features of some of the more established monitoring tests.

Cytogenetic tests

By far the greatest body of human genetic monitoring experience has been with cytogenetic tests. Cytogenetic abnormalities are associated with cancer in several ways. Several human cancers are

associated with specific chromosomal rearrangements or deletions. For example, chronic myeloid leukemia cells contain a terminal deletion in chromosome 22 (Philadelphia chromosome)[5] and a reciprocol translocation between chromosomes 8 and 14 is observed in Burkitts lymphoma.[6] In three inherited human syndromes predisposing to cancer, cells show elevated levels of chromosomal abnormalities: Bloom's syndrome,[7] Ataxia telangectasia,[8] and Franconi's anemia.[9] Among the short term tests for carcinogens, assays for chromosome damage have been remarkably accurate.[10]

Cytogenetic analysis for human monitoring is usually done with peripheral lymphocytes using structural damage (chromosome breaks and exchanges) and sister chromatid exchange as the end points. The technical aspects of cytogenetic monitoring have recently been reviewed by Galloway and Tice,[11] and by Bloom.[12] The human peripheral lymphocyte is a particularly attractive cell with which to do cytogenetic analysis because its lifetime in the circulation is several years.[13-16] In addition, it can tolerate certain types of genetic damage for long periods.[17] It is easily and safely obtained and reliably cultured by well established methods. The systematic classification of structural aberrations[18,19] provides a uniform standard for scoring. The sister chromatids of metaphase chromosomes can be differentially stained permitting the observation of reciprocal exchanges of homologous regions (sister chromatid exchange or SCE).[20]

Both structural aberration and SCE analysis have been performed with populations exposed to a variety of agents (reviewed by Sorsa, et

al.[3]). Increased rates of chromosome damage have been observed in populations exposed to all the agents classified as known or suspect human carcinogens by the IARC for which human chromosome studies have been done.[21] Structural aberrations have been shown to be a sensitive response to certain exposures. Exposures to gamma radiation at doses below the internationally accepted limit of 5 rem per year have produced increased chromosome aberration rates in a population of nuclear dockyard workers.[22]

Small increases in both structural aberrations and sister chromatid exchanges can be detected in relatively small populations. As figures 2a and b indicate a 1% increase over a 2% base rate of structural aberrations could be detected in a population of 48 subjects[23] and an increase of 1 SCE per cell over a background of 5 SCE per cell could be detected in about 35 subjects (Whorton, pers. commun.).

Sperm Tests

The analysis of sperm is attractive in genetic monitoring because of the opportunity to observe effects in germ cells. Currently three types of endpoints have been evaluated as indicators of genotoxic exposure: sperm count, morphology, and the frequency of Y bodies. These methods have recently been reviewed by Wyrobek et al.[24]

Reduction in sperm count is a response to many different conditions and is not a specific response to mutagenic agents.

Nevertheless it is a worthwhile parameter to ascertain because in is a good indicator of the general quality of the sample being evaluated. Furthermore, in at least the case of dibromochloropropane (DBCP) dramatically reduced sperm count was the presenting effect which indicated a health hazard in occupational exposure.[25] Sperm counts can be quickly and easily determined by standardized methods.[26] Although sperm counts vary, analysis of sperm for morphological abnormalities is based on the observation that head morphology appears to be under strict genetic control in the mouse and that exposure to mutagenic agents leads to changes in morphology.[27] Human sperm can be classified into several morphological types and the distribution of different types determined. Wyrobek et al,[24] have developed a standardized protocol for scoring sperm morphology which can effectly stabilize variation in scoring over time and even among scorers.

The YFF test is based on the observation that the human Y chromosome stains so intensely with quinarine that it can be seen in interphase cells as a bright spot.[28] About 40-50% of sperm are observed to contain a fluorescent spot or Y body (YF). About 1% of sperm contain two Y bodies (YFF).[29] The second Y body has been proposed to be a second Y chromosome[29] although this point remains to be established. The putative cause of the appearance of a second Y chromosome is non-disjunction occurring at meiotic anaphase II.[30] Kapp and Jacobsen evaluated the stability of the frequency of the second Y body and its response to exposure to X irradiation and adriamycin. The YFF frequency increased from 1% to 3-5% starting about 21 days following the onset of these exposures.

The ability of these sperm tests to detect occupational exposures has been demonstrated in a limited number of cases. Sperm count was reduced in workers exposed to DBCP[25] and lead.[31] DBCP was also associated with increased YFF frequency.[32] Abnormal sperm morphology has been associated with exposure to lead[31] and carbaryl.[33]

Wyrobek et al,[33] have estimated sample size requirements for all three sperm tests. Mean increases over control values of .25 standard deviation units could be detected with p = .05 and a power of 90% with sample sizes calculated to be 214, 26, and 41 individuals for sperm count, morphology and YFF respectively. Except for sperm count these sample sizes requirements are similar to those for cytogenetic studies.

Body fluid analysis

The detection of mutagenic substances in body fluids is an indication the ingestion, distribution and excretion of a substance or substances capable of producing genetic damage. Urine samples are tested for the presence of mutagens using an in vitro short term test. The Ames/Salmonella assay[34] has been the most commonly used although tests for point mutation or cytogenetic effects using mammalian cells or other organisms can be used.[35,36] Similarly blood or other body fluids could be used as sample sources. Although urine can be tested directly it is common practice to concentrate a non-polar fraction from the urine using XAD-2 resin extraction.[37] This serves the dual purpose of concentrating mutagens and of removing histidine from the

material tested. Low histidine levels interfere with the Ames/Salmonella assay.[38] The original concentration technique[37] is not optimal for concentrating all mutagens. Consequently it is prudent to conduct preliminary tests with urine from treated animals or human urine to which test agents have been added to insure that the procedure efficiently concentrates the materials of interest in a particular study.

The detection of mutagens in urine is a non-specific procedure since any agent present will be detected. For situations in which exposures are poorly characterized this is an advantage since the technique is not dependent on the detection of specific agents. If mutagenic activity is found additional urine samples can be subjected to chemical analysis to identify the mutagen and determine its source.[38]

On the other hand, the non-specific nature of the assay makes it susceptible to interference from other sources of exposure, for example, cigarette smoking.[37,39] Proper experimental design, as described below can adequately control for other sources of variation.

The analysis of urine has detected mutagens in several occupational exposures[3] including oncology nurses and pharmacists involved in preparing and administering cancer chemotherapy drugs as reviewed by Falke in this volume.[40]

Other assays

Several other assay systems are in various stages of development at this time which are suitable or potentially useful for monitoring human populations. Most notably, a technique for quantitating specific locus mutation in peripheral lymphocytes is now available.[41] Techniques exploiting new immunological methods and DNA analysis are being used in the development of new assays which are reviewed by Baan et al.[42] in this volume.

IV. Interpretation

In interpreting results of these assays it must be clearly understood that the biological events detected are not adverse health outcomes in themselves. While biological events such as chromosome breakage are associated with exposure to carcinogens, the mechanistic relationship between these events and the induction of neoplasia is not currently understood. In addition all of these events are known to occur in the general population at a finite rate. Recognition of these facts has important implications for the use and interpretation of these tests as well as the way in which results are presented to study participants and sponsors.

The purpose of monitoring tests is to detect biologically significant exposures at an early time point. The lack of a mechanistically understood relationship between the monitoring endpoints and adverse health effects makes results of these studies unsuitable as a basis for a quantitative risk assessment. For example, one cannot extrapolate from a 2% increase in chromosome

breakage rate in a population of workers to a precise quantitative increase in risk of some form of cancer.

Furthermore, monitoring tests should be interpreted on the basis of population rather than individual results. This is true for two reasons. The first is, again, the lack of a mechanistic understanding of the relationship between the test endpoint and the adverse health outcome. The second is the range of normal variation among individuals in the general population. There is currently no basis for concluding that an individual in a group under study with the greatest level of genetic damage is at the greatest risk of experiencing an adverse health outcome. Rather the view that should be taken is that an increase in the mean level of damage experienced by a population with a relatively homogenous exposure, as compared to a suitable control group, indicates a significant level of exposure. This exposure implies an increased risk which cannot be precisely quantified. In the absence of more specific data on individual exposure the risk should be considered the same for all individuals in the population regardless of their individual test results.

A corollary is that data resulting from monitoring studies cannot be used to identify individuals who are hypersusceptible to the effects of mutagen or carcinogen exposure. On the basis of monitoring data alone there is no way to differentiate between hypersusceptibility and elevated exposure. Furthermore, elevated responses in short-term tests for genetic damage in individuals have not been shown to imply an increased predisposition to cancer.

For these reasons the results reported to individual participants in monitoring studies should be based on the population rather than their individual findings. Similarly, actions taken in response to monitoring results should be at the population level. Removal of only those individuals with the most abnormal test results from the hazardous environment might well leave other individuals, whose actual risk is just as great, in continued jeopardy. Measures which reduce exposure for the entire population would be more appropriate.

The same characteristics of genetic monitoring endpoints which influence the interpretation of results, as described above, also influence the design of monitoring studies. The comparison of a population under study to a control group is imperative for several reasons. Since several of the endpoints observed are general responses to DNA damaging agents they may be affected by many genetically active substances in the environments of individual subjects. Cigarette smoking, for example, is a common source of exposure to carcinogens which has been shown to increase rates of chromosome damage,[43] sister chromatid exchange[44] and urinary mutagen excretion.[37] Variability due to exposure to other common mutagens can be controlled by matching participants in exposed groups with control subjects on the basis of sex, age, lifestyle factors such as smoking or alcohol consumption. Use of medications and other highly individual factors may be difficult to use as matching characteristics but it is important to obtain information on these exposures so that their influence can be determined in interpreting results. By matching individuals in exposed and control groups the general

characteristics of the populations can be made similar so that comparison of the populations will focus on the specific exposure differences which are the purpose of the study. The size of the population to be studied is a critical design factor. As with any experiment which seeks to detect an increase over a range of normal variation, consideration must be given to the degree of abnormality which one seeks to detect and the statistical power required to detect it. In practical terms this will influence the size of the population to be studied. Given historical background rates for a specific test and the acceptable limits of false positive (alpha) error and false negative (beta) error the population sizes required to detect specific increases in abnormality can be calculated.[23]

Experimental variability due to laboratory factors must be controlled. Some tests require extensive manipulation of samples obtained from subjects in the laboratory. For example, cytogenetic analysis requires <u>in vitro</u> culture of peripheral lymphocytes. Differences in cell culture methods may alter chromosome aberration rates.[45] Laboratory factors can be controlled by simultaneously obtaining and processing samples from matched control and exposed subjects. Consistant use of the same techniques and materials throughout the study is also an important factor in controlling variation. Because evaluation of many of the endpoints used in monitoring studies requires the subjective judgement of the investigator, scoring should be done on coded samples on a blind basis. In some cases uniformity and objectivity of scoring may be aided by the repeated use of laboratory reference standards.[23]

Variation due to both subject characteristics and laboratory factors may be further controlled by sampling the study populations repeatedly at regular intervals. This both increases the sample size and diminishes the impact of temporal variables such as episodic outbreaks of viral infection or sporadic abnormal values of unknown cause.

Not all carcinogens interact with the genetic apparatus in the same manner. One agent may evoke a particular response while another agent evokes a different response. A monitoring study which relies on a single test may fail to detect an exposure to which it lacks sensitivity. By using a battery of tests which detect different types of events in different tissues the probability of detecting an effect should be greatly increased. At the same time a negative finding in a battery of tests can be accepted with greater confidence than if only one test was performed.

In summary, although there are many variables associated with human monitoring tests resulting from individual differences and laboratory techniques appropriate experimental design can control them. As a result statistically valid inferences can be drawn as to the experience of specific exposure groups. Monitoring tests based on indicators of genetic damage or mutagen exposure can be used to detect population exposures to hazardous agents or environments.

V. Implimentation of design features: A study of formaldehyde exposure in pathologists

A study conducted by our laboratory was designed so as to take into account the considerations discussed in the preceding section. The study involved twelve individuals employed on the Autopsy Service of a major medical center hospital where they were exposed to formaldehyde. In addition seven other individuals with other responsibilties in the Pathology Department involving formaldehyde exposure were also studied. A battery of tests were performed including environmental measurements to determine formaldehyde levels. In so far as possible animal tests analagous to the human monitoring studies were performed to determine the potential activity of formaldehyde and to optimize methods. The human tests and their animal analogues are presented in figure 3.

Each subject was matched with a control subject on the basis of age, sex, customary use of tobacco, alcohol, and recreational drugs. Information collected on caffeine consumption, medications, illnesses and non-occupational exposures to potential mutagens was used in evaluating results. Exposed and control subjects were sampled simultaneously to control for laboratory factors and subjects were sampled three times at intervals of two to three months.

Environmental measurements in the autopsy area indicated that exposures occurred in transient episodes during specific activities such as sectioning, tissue examination or autopsy conferences. The time weighted average exposure was estimated to be in the range of 1 ppm.[46]

Cytogenetic evaluation detected no differences between the exposed and control populations for structural aberrations, SCE's or growth kinetics (Tables 2,3). Structural aberration analysis in $B_6C_3F_1$ mice detected a dose dependent increase in exchange aberrations (centric fusions)(Table 4) between 5 and 20 mg/kg body weight. No increases in micronuclei were observed in mouse bone marrow at doses up to 40 mg/kg body weight. Sperm tests for count, morphology and YFF detected no increased abnormality in humans and no increases in mouse sperm abnormality were seen. The urine analysis of human subjects detected no increase in mutagenicity although a clustering of subjects with urine extracts toxic to Salmonella was seen in the exposed group (Table 5). No mutagenic activity was seen in mouse urine. Studies are still in progress to determine whether adducts to hemoglobin can be detected.

In summary human exposure to the levels of formaldehyde described did not produce any detectable genetic effects. The animal studies suggest the possibility that much higher levels of exposure could produce chromosome damage. The toxic effects seen with human urine could be associated with formaldehyde exposure although that point remains unresolved.

The population studied in this case was relatively small. In addition, formaldehyde is highly reactive with tissues and rapidly metabolized.[47] It is possible that genetic damage could be produced in cells of the respiratory epithelium without detectable effects occurring in the more distant cells observed here. It would be

premature to conclude that human populations exposed to a time weighted average exposure of 1 ppm are safe from genetic hazards.

VI. Future Research Needs

Several research needs must be met in order to implement human monitoring as an established procedure and to fully realize its potential. The most fundamental problem is to better understand the relationship between the endpoints used in monitoring and the adverse health outcomes which they are intended to predict. Our current lack of understanding is the major reason for the limitations imposed in interpreting test results as discussed earlier. It is also the weakness most often cited as a reason for the reluctance of corporations, unions or other groups to participate in monitoring studies. The rapid progress now being made in understanding mechanisms of carcinogenesis may help to clarify the significance of some forms of genetic damage. Studies which specifically examine the relationship between genetic damage and tumor induction in animals should be of great interest.

A better understanding of the sensitivity and specificity of monitoring tests in human populations is needed. This requires the participation of many populations with exposures to a variety of agents as well as to different exposure levels. If the specificities of different tests are better defined, batteries of fewer tests taylored to specific situations can be used. This would reduce costs and allow monitoring tests to be conducted on a more routine basis.

Similarly, once the sensitivities of specific tests to particular agents is established decisions regarding both test selection and population sample size can be made on a rational basis.

Continued efforts to develop new tests are entirely appropriate. It is by no means clear that the tests currently available measure effects most relevant to the neoplastic process. It may be that tests detecting events at the molecular level, such as adduct formation or single strand breakage, or gene rearrangement would prove to be better predictors of neoplasia. In addition the rapid development of methodologies in molecular genetics and immunology may offer levels of specificity and sensitivity not available today. New tests are also needed which detect effects in specific tissues, particularly those at the site of exposure to direct acting agents. These sights would include the respiratory and digestive tracts and the skin. The lack of sensitivity of current tests to formaldehyde as seen in our study points to the need for such tests in order to clarify the hazards associated with exposure. The emuneration of micronucleii in exfoliated cells described elsewhere in this volume[48] is an interesting technique which addresses this problem.

These research needs cannot be met without the willing cooperation of populations of human subjects who are sustaining exposures to potential or known carcinogens in the course of their work or in medical treatment. Studies of sufficient scope and sophistication to meet the objectives of this research will not be possible without the cooperative participation of private industry,

organized labor, governmental agenices and the research community. In order to achieve the level of trust and cooperation needed to conduct meaningful research several social, ethical and legal issues related to the conduct of studies and the interpretation and use of results need to be addressed and agreed upon The appropriate use of monitoring data for scientific, regulatory and legal purposes is an area of particular concern.

The monitoring of human populations for genetic damage has the potential improve our ability to detect health hazards and prevent or reduce the risk of workplace-exposure-related cancer. In the long run this can reduce costs related to illness. It is possible that the costs of exposure control can also be reduced by better establishing the sources of significant exposure and by understanding the human response to exposure. However, its greatest promise is in the protection of human health through the early detection and prevention of disease processes resulting from mutagenic exposure.

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Legends for Figures

Figure 1. Biological consequences of exposure to mutagenic agents. Exposure produces DNA damage which is resolved by cell death, repair, or mutation. These effects are immediately observable and form the basis for short term genetic monitoring tests. Mutation contributes to the process leading ultimately to cancer. Also mutation in germ cells or embryonic cells can produce reproductive effects. These adverse health outcomes become apparent only after a lag period. As a result they are not as easily detected as the immediate effects. Furthermore, it is more difficult to make an association between the health effects and an exposure than between the immediate effects and exposure.

Figure 2. Sample size requirements for cytogenetic monitoring studies. Panel A. Structural aberration analysis. The number of subjects required to detect indicated increases over a control aberration rate of 2 per 100 cells is shown when the number of cells scored is 100 per subject (O) or 200 per subject (□). The error limits used are $\alpha = .05$; $\beta = .20$ Data are from.[23] Panel B. Sister chromatid exchange analysis. The number of subjects required to detect an indicated increase in SCE per cell is shown when the control rate is 5 SCE per cell (O) or 11 SCE per cell (□) and 25 cells per subject are scored. The error limits used are $\alpha = .05$ and $\beta = .05$. This analysis was made by E.B. Whorton, Jr. using data supplied by R.R. Tice (personal communication).

Figure 3. Human monitoring tests used in the study of formaldehyde exposure in autopsy service workers and equivalent tests performed in animals. (Reprinted from reference [21] with permission).

Figure 1

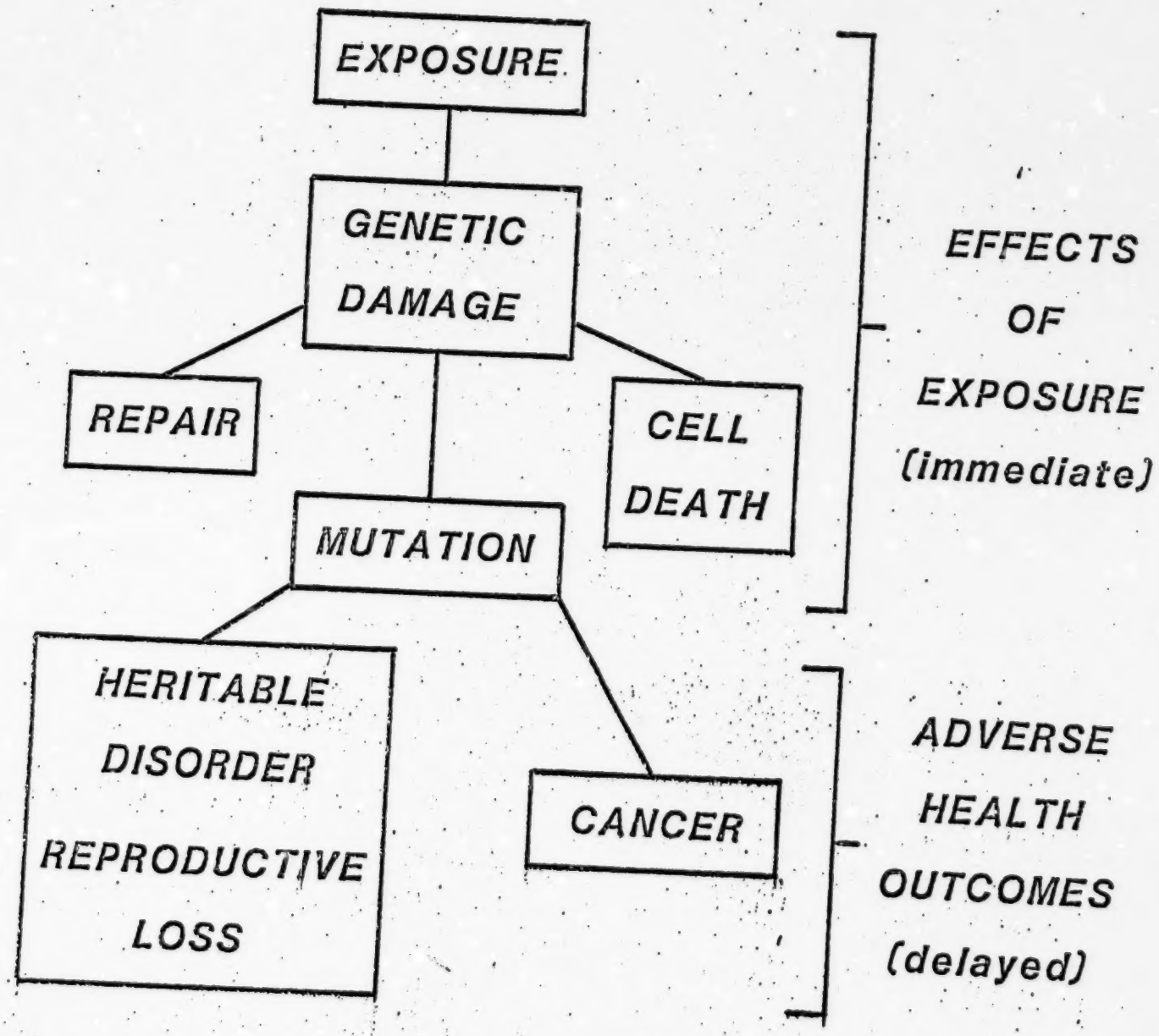
EXPOSURE
GENETIC DAMAGE
REPAIR
CELL DEATH
MUTATION
HERITABLE DISORDER REPRODUCTIVE LOSS
CANCER
EFFECTS OF EXPOSURE (immediate)
ADVERSE HEALTH OUTCOMES (delayed)

Figure 2A

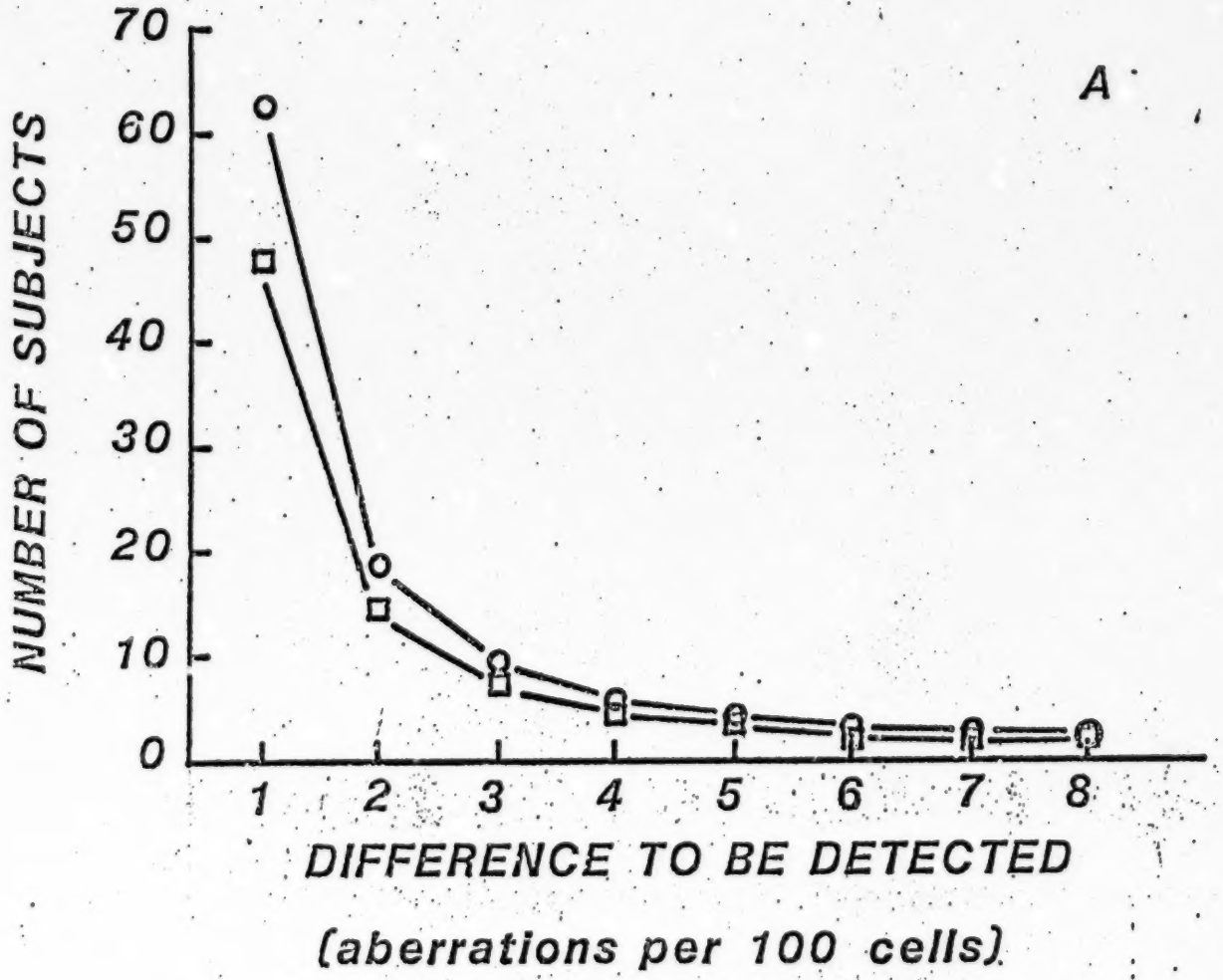
A
NUMBER OF SUBJECTS
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
DIFFERENCE TO BE DETECTED
(aberrations per 100 cells)

Figure 2B

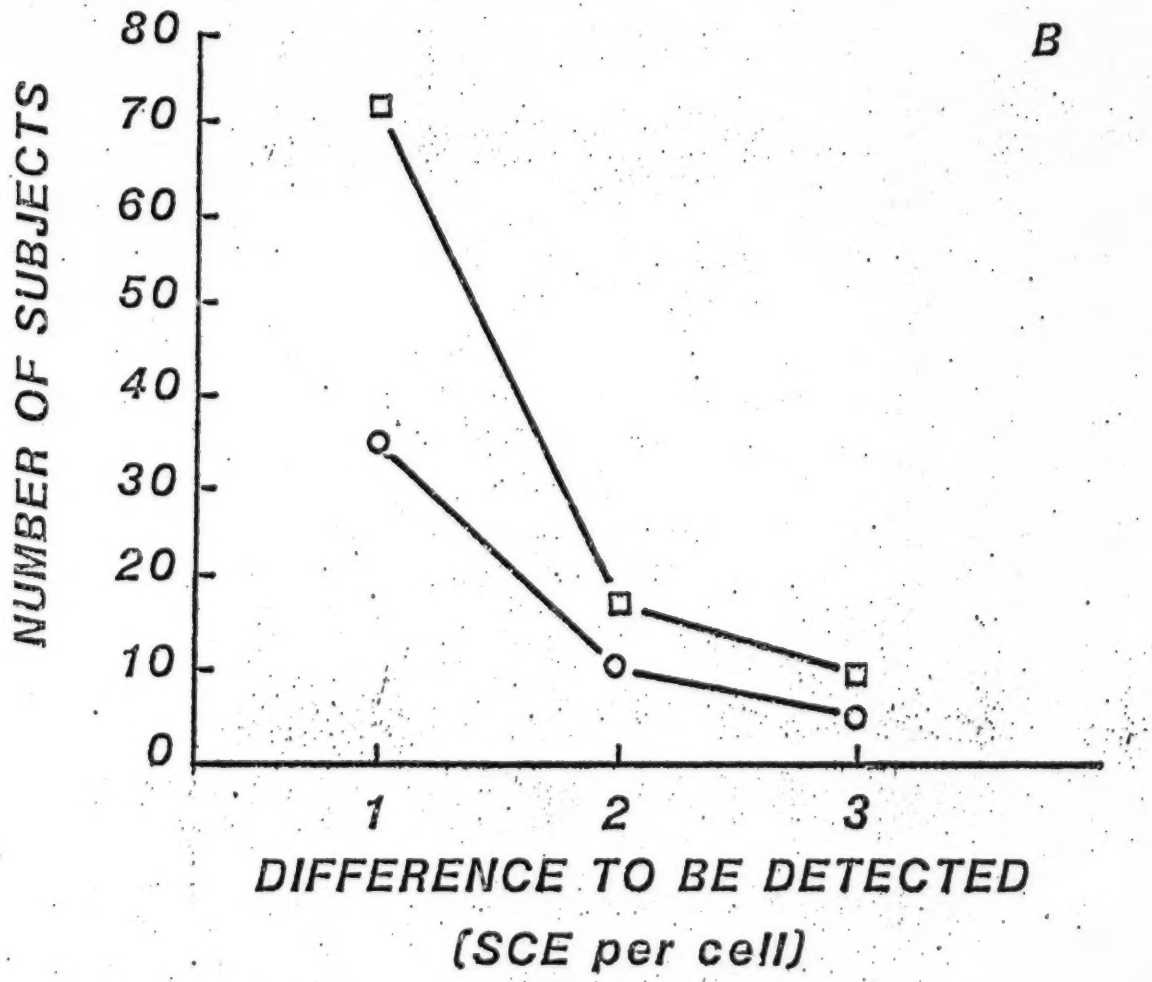
B
NUMBER OF SUBJECTS
80
70
60
50
40
30
20
10
0
1
2
3
DIFFERENCE TO BE DETECTED
(SCE per cell)

Table 1. Tests available for monitoring human populations for genetic damage

Somatic cell effects.

Test	Cell or fluid	biological level
Structural		
Chromosome aberration	lymphocyte	chromosomal
Sister Chromatid		
Exchange	lymphocyte	chromosomal
6-Thioguanine		
Resistance	lymphocyte	gene mutation
Body fluid		
Analysis	urine	gene mutation
Macromolecule		
Alkylation	erythrocyte (hemoglobin) lymphocyte (DNA)	molecular-adduct formation
Alkaline		
Filter Elution	lymphocyte	molecular- (DNA single strand breaks alkali labile sites, cross-links)

Germinal cell effects.

Test	Cell or fluid	biological level
Sperm Count	semen	general toxicity including mutagenicity
Sperm Morphology	sperm	gene mutation (?)
Y Fluorescent Body Frequency (YFF)	sperm	chromosome non-disjunction(?)

Figure 3

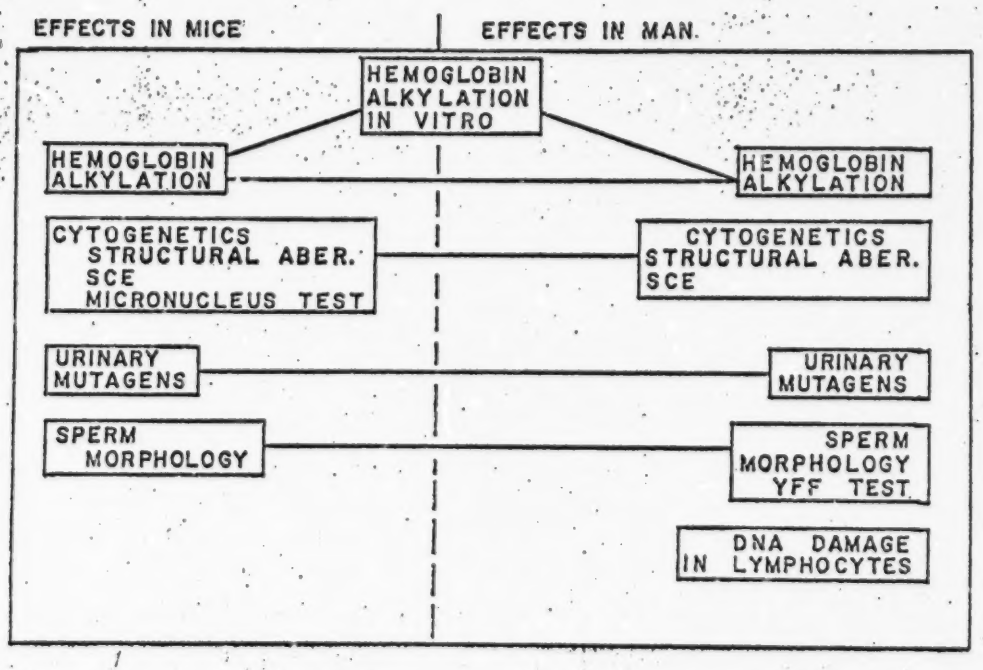
EFFECTS IN MICE
EFFECTS IN MAN.
HEMOGLOBIN ALKYLATION IN VITRO
HEMOGLOBIN ALKYLATION
HEMOGLOBIN ALKYLATION
CYTOGENETICS STRUCTURAL ABER. SCE MICRONUCLEUS TEST
CYTOGENETICS STRUCTURAL ABER. SCE
URINARY MUTAGENS
URINARY MUTAGENS
SPERM MORPHOLOGY
SPERM MORPHOLOGY YFF TEST
DNA DAMAGE IN LYMPHOCYTES

TABLE 2

HUMAN EXPOSURE TO FORMALDEHYDE

STRUCTURAL CHROMOSOME ABERRATIONS

Group	High		Low		Total	
	Exposed	Control	Exposed	Control	Exposed	Control
n subjects	12	13	7	7	19	20
n samples	30	30	20	21	50	51
n cells	5591	5991	3995	4080	9586	10071
Gaps	38	50	26	32	64	82
Breaks	66	100	58	85	124	185
Exchanges	3	3	3	3	6	6
Other	4	9	2	4	6	13
AB/100[1]	1.31	1.87	1.58	2.25	1.42	2.03
AB/100[2]	1.98	2.70	2.23	3.04	2.09	2.84

[1] excluding gaps

[2] including gaps

TABLE 3

FORMALDEHYDE EXPOSURE STUDY

SISTER CHROMATID EXCHANGE

Group	High		Low		Total	
	Exposed	Control	Exposed	Control	Exposed	Control
n subjects	4	4	6	6	10	10
n samples	7	8	8	7	15	15
n cells	201	240	222	210	423	450
% 1st Division	69.8	68.0	78.9	71.4	75.2	70.7
% 2nd Division	31.0	30.5	20.9	24.8	24.9	27.1
% 3rd Division	0.5	1.75	1.5	4.1	0.3	2.9
SCE Per Cell	6.77	6.02	6.55	6.68	6.64	6.42
± S.D.	0.96	0.47	1.13	0.67	1.01	0.67

TABLE 4

STRUCTURAL CHROMOSOME ABERRATIONS IN

MICE TREATED WITH FORMALDEHYDE

Treatment / Dose	Water	Formaldehyde				Cytoxan
		5mg/kg	10mg/kg	20mg/kg	40mg/kg	
n animals	10	10	10	10	10	4[3]
n cells	493	400	500	500	500	63
aneuploid cells	24	26	66	43	51	9
gaps	7	3	0	0	1	2
breaks	2	9	2	2	1	66[4]
centric fusions	8	13	24	37	16	0
AB/100[1]	2.0	5.5	5.2	7.8	3.4	105
AB/100[2]	3.4	6.25	5.2	7.8	3.6	108

[1] excluding gaps

[2] including gaps

[3] no metaphase cells observed in 6 of 10 animals treated

[4] also 3 rings, 1 triradial, 5 severely damaged, 1 moderately damaged

TABLE 5

FORMALDEHYDE EXPOSURE STUDY

MUTAGENICITY OF URINE CONCENTRATES

Group	Unit	n	Result			
			Neg.	Pos.	Toxic	Quest.
High Exp.	sample	36	21	2	12	1
	subject	12	3	1	7	1
High Cont.	sample	36	28	1	5	2
	subject	13	9	0	3	1
,ow Exp.	sample	19	11	0	4	4
	subject	7	3	0	2	2
Low Cont.	sample	21	18	3	0	0
	subject	7	6	1	0	0